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Applicant: Charles T. Esmon and Jun Xu

Serial No.: 09/139,425 Art Unit: 1636

Filed: August 25, 1998 Examiner: W. Sandals

For: *"TARGETING OF MOLECULES TO LARGE VESSEL ENDOTHELIUM USING PCR"*

Assistant Commissioner for Patents
Washington, D.C. 20231

APPEAL BRIEF

Sir:

This is an appeal from the final rejection of claims 5, 6, 12, 13 and 16-20 in the Office Action mailed October 4, 2001, as maintained in the Advisory Action mailed on April 19, 2002, in the above-identified patent application. In the Advisory Action mailed on April 19, 2002, the Examiner stated that claims 1-4 and 7-11 are allowed and claims 14, 15, and 21-25 objected to as dependent upon rejected independent claims. An Amendment to rewrite these claims in independent form accompanies this Appeal Brief. A Notice of Appeal was mailed on February 27, 2002. A request to charge our deposit order in the amount of $215.00 for the filing of this Appeal Brief for a small entity is also enclosed. Submitted with this Appeal Brief is a Petition for an Extension of Time, along with the required fee for a small entity, to extend the period for response one month, to and including May 27, 2002. It is believed that no additional fee is

required with this submission. However, should an additional fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-1868.

(1) REAL PARTY IN INTEREST

The real party in interest of this application is Oklahoma Medical Research Foundation, the assignee.

(2) RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to appellant, the undersigned, or appellant's assignee which directly affects, which would be directly affected by, or which would have a bearing on the Board's decision in this appeal.

(3) STATUS OF CLAIMS ON APPEAL

Claims 1-25 are pending. Claims 1-4 and 7-11 are allowed and claims 14, 15, and 21-25 are objected to. Claims 5, 6, 12, 13, and 16-20 are on appeal. The text of each claim on appeal, as pending, is set forth in an Appendix to this Appeal Brief.

(4) STATUS OF AMENDMENTS

An amendment after final rejection was mailed on February 4, 2002. In the Advisory Action mailed April 19, 2002, the Examiner indicated that this amendment would be entered upon the timely submission of the Appeal Brief. The Advisory Action states that claims 1-4 and 7-11 are allowed; claims 14, 15, and 21-25 are objected to; and claims 5, 6, 12, 13, and 16-20 stand rejected. An appendix sets forth the claims on appeal. Accompanying this Appeal Brief is an amendment rewriting the objected to claims in independent form, including all of the limitations of the claims from which they depend.

(5) SUMMARY OF THE INVENTION

The claimed method is directed to selectively delivering molecules to the nucleus of endothelial cells of the large vessels, by administering a conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) and the molecule to be delivered to large vessel endothelial cells. The conjugate binds to the receptor, the conjugate is endocytosed, and the conjugate thereby delivers the molecule to the cytoplasm or to the nucleus of the large vessel endothelial cells (see claim 1 as originally filed and Examples in specification). The conjugate may be delivered by directly contacting the endothelial cells of large vessels with the conjugate or by catherization within blood vessels formed by the endothelial cells (page 10, lines 15-18). The conjugate may be administered to an individual in need of treatment or diagnosis (page 9, lines 22-26).

The conjugate includes (1) an agent binding to endothelial protein C receptor (EPCR), protein C, activated protein C, antibodies reactive with EPCR, or fragments thereof binding to EPCR, and (2) a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label (page 4, lines 8-11). The conjugate may be a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin (page 2, lines 12-17). The conjugate may also be formed as a recombinant molecule based upon an antibody to EPCR (page 2, line 16). The molecule may be a gene or a cDNA controlled by a promoter expressed in the nucleus of an endothelial cell, a drug other than nucleic acids and proteins (page 6, line 26), a protein or a transcription factor (page 7, lines 16-17). The coupling means may be a positively charged polymer or molecule; or

straptavidin-biotin (page 9, lines 1-2). The conjugate may comprise a chimeric antibody which binds to EPCR and to the molecule to be delivered (page 8, lines 15-17).

(6) ISSUES ON APPEAL

The issues presented on appeal are:

(1) whether claims 5, 6, 12, and 16-18 are enabled as required by 35 U.S.C. § 112, first paragraph; and

(2) whether claims 13, 15, 19, and 20 were properly rejected under 35 U.S.C. § 102(b) as lacking novelty over U.S. Patent No. 5,254,532.

(7) GROUPING OF CLAIMS

The claims do not stand or fall together. The claims can be grouped as follows: Claims 5, 6 and 16-20 further define the molecule to be delivered. Claim 12 defines the cells to be targeted by the method. Claim 13 is directed to a conjugate of an agent binding selectively to endothelial protein C receptor selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin. Reasons for this grouping and arguments for the separate patentability of these groups of claims are provided below.

(8) ARGUMENTS

(a) The Claimed Invention

The claimed method and composition are directed to conjugated molecules that bind to the Endothelial Protein C Receptor (EPCR) of endothelial cells and are subsequently internalized. Each conjugated molecule consists of at least two parts: (1) the EPCR binding portion (the binding agent) and (2) the molecule to be targeted to, and internalized by, the endothelial cell. It is well established that agents that selectively bind to EPCR include protein C, activated protein C, and EPCR antibodies and EPCR antibody fragments. Localization of the conjugate within the endothelial cell (i.e. the nucleus or the cytoplasm) is dependent upon this agent. For example, molecules conjugated to Activated Protein C, or antibodies, will be transported to the nucleus. Molecules conjugated to Protein C will be transported to the cytoplasm.

The molecules conjugated to the agent may be nucleic acids, proteins, diagnostic agents, or drugs. Molecules that are required to be expressed in order to be effective in the endothelial cell will be translocated to the nucleus, for example, *via* Activated Protein C or an antibody, where the proper cellular machinery may be utilized to transcribe the nucleic acid.

The molecule is coupled to the EPCR binding agent. Coupling means are well established in the art and include covalent or ionic interactions; chemical coupling, for example, *via* succinic anhydride; chimeric proteins or protein fusions. Indirect binding may be accomplished *via* an intermediate molecule like streptavidin or biotin, or via a positively charged polymer like lysine, pyrrole, or chitosan.

(b) Rejections Under 35 U.S.C. § 112

Claims 5, 6, 12 and 16-18 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled.

i. The Legal Standard.

The Court of Appeals for the Federal Circuit (CAFC) has described the legal standard for enablement under § 112, first paragraph, as whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*See, e.g., Genentech, Inc. v. Novo Nordisk A/S*, 108 F3d at 165, 42 USPQ2d at 1004 (quoting *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993); See also *In re Fisher*, 427 F.2d at 839, 166 USPQ at 24; *United States v. Telectronics, Inc.*, 857 F.2d 778 (Fed. Cir. 1988); *In re Stephens*, 529 F.2d 1343 (CCPA 1976)). The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation (*M.I.T. v. A.B. Fortia*, 774 F.2d 1104 (Fed. Cir. 1985)). In addition, as affirmed by the Court in *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524 (Fed. Cir. 1987), a patent need not teach, and preferably omits, what is well known in the art.

Whether the disclosure is enabling is a legal conclusion based upon several underlying factual inquiries. See *In re Wands*, 858 F.2d 731, 735, 736-737, 8 USPQ2d 1400, 1402, 1404 (Fed. Cir. 1988). As set forth in *Wands*, the factors to be considered in determining whether a claimed invention is enabled throughout its scope without undue experimentation include the quantity of experimentation necessary, the amount of direction or guidance presented, the

presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims. In cases that involve unpredictable factors, "the scope of the enablement obviously varies inversely with the degree of unpredictability of the factors involved." *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation 'must not be unduly extensive.' *Atlas Powder Co., v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984).

ii. Factual Analysis of Claims 5-6, 12, and 16-18 under 35 U.S.C. § 112, first paragraph.

The pending method claims are directed to selectively delivering molecules to the nucleus of endothelial cells. The specification is clearly enabled for the *delivery* of the claimed molecules (conjugates of an agent selectively binding to endothelial protein C receptor and the molecule to be delivered). The Examiner has consistently referred to "anecdotal reports of patients being helped by gene therapy". However, rejected claims 5 and 6 are simply directed to the delivery of conjugated molecules (nucleic acids conjugated to the endothelial protein C receptor binding agent) to endothelial cells. The appellants have provided Examples, that when taken in combination with the state of the art at the time of filing the present application, clearly enable claims 5 and 6. Example 3 and Figure 1 illustrate the efficiency of reporter gene transfer to the nucleus of endothelial cells. The reporter gene is complexed with anti-EPCR monoclonal antibodies *via* poly-L-lysine and targeted to the endothelial cell receptor. Once bound to the receptor, the complex is internalized by the cell and transported to the nucleus for expression of

the reporter gene. Figure 1 shows a consistently higher level of reporter gene expression in cells that were transfected with reporter gene DNA complexed with anti-EPCR monoclonal antibodies as compared to cells being targeted *via* non-specific monoclonal antibodies (an approximately 25-fold increase over the control in one sample). Given the positive charge of poly-L-lysine, one of ordinary skill in the art would reasonably expect and realize, in view of Example 3, that nucleic acids (negatively charged) readily form complexes with the poly-L-lysine coupled to the EPCR-monoclonal antibodies. Once bound by the endothelial cell receptor, these complexes are efficiently taken up by the endothelial cells harboring the receptor, transported to the nucleus, and the genes within the complex expressed.

Claims 12 and 16-18 are directed to the administration of the conjugate to cells of an individual in need of treatment or diagnosis (claim 12), and the *in vitro* treatment of endothelial cells of large vessels or by catherization to endothelial cells (claims 16-18). The appellants have submitted data in the form of a reference by Baumgartner *et al.* (*Circulation*, March 31, 1998; see After Final amendment and response mailed on February 4, 2002), to show the state of the art with regard to treating endothelial cells at the time of filing the present application, August 1998. Baumgartner *et al.* demonstrates therapeutic intramuscular gene transfer to endothelial cells in need of treatment using plasmid DNA encoding an endothelial cell mitogen. Baumgartner explicitly teaches successful gene therapy to endothelial cells. Baumgartner presents results from a phase 1 trial, "unanimously approved by the Recombinant DNA Advisory Committee and the U.S. Food and Drug Administration" and used to study new chemotherapeutic agents administered to human subjects. The data presented shows gene

expression *at the protein level* as a transient peak of gene product in the systemic circulation one to three weeks after gene transfer (see Figure 1, and description at page 1116 of Baumgartner).

The appellants respectfully submit that the predictability, or lack thereof, in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. What is known in the art provides evidence as to the question of predictability. *In re Marzocchi*, 439 F.2d 220, 223-224, 169 USPQ 367, 369-370 (CCPA 1971). As taught by Baumgartner *et al.*, the transfer of naked DNA *to endothelial cell* wherein the DNA is stably expressed, had been accomplished with success. The Examiner has continually argued that there is no conclusive evidence of successful treatment of human disease using gene therapy. The Examiner appears to have not even considered the data presented by Baumgartner *et al.*, wherein nonhealing ischemic ulcers and/or rest pain due to peripheral arterial disease are healed or markedly improved, including successful limb salvage in three patients using gene therapy directed to endothelial cells.

In view of the specification, the Examples therein, and the references that have been previously submitted (in particular, the Baumgartner *et. al.* reference), one of ordinary skill in the art would have reasonably expected that the claimed method, using molecules conjugated to agents that selectively bind to EPCR, could be effectively used *for the delivery* of nucleic acid to endothelial cells in need of treatment. The examiner has provided no evidence that one could not practice the claimed method. The examiner has instead relied on conclusory statements without putting forth specific reasons describing why the claims are not enabled by the specification. The

patent examiner cannot just assert that the application is not enabled. As stated in In re Marzocchi at 439 F.2d 220 (CCPA 1971:

> [I]t is incumbent upon the Patent Office, whenever a rejection on this basis is made [, enablement under § 112, first paragraph], to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the Appellant to go to the trouble and expense of supporting his presumptively accurate disclosure.
>
> *Id.* at 224.

As an illustration of the examiner's burden, in *Ex parte Goeddel*, 5 U.S.P.Q.2d 1449 (1985), the Board of Patent Appeals overturned an examiner's rejection based on lack of enablement. The invention at issue claimed therapeutically active fraction of a polypeptide consisting essentially of the amino acid sequence of a mature human leukocyte interferon. Although the examiner cited *Jackson* for the proposition that the art was uncertain, the Board found that, where "appellants in a comprehensive and detailed disclosure have set forth the manner by which the claimed leukocyte interferons may be obtained," one "skilled in this art palpably would have no difficulty following appellants' instructions in order to realize the claimed product staring with known and available precursors." Accordingly, the vague citation to *Jackson* did not "rebut appellants' extensive and well reasoned arguments that the disclosure in

this case is adequate . . . . Mere broad generalizations and allegations are insufficient for holding of non-enablement."

In this case, the examiner has not even bothered to respond to the evidence and why it is not considered sufficient to overcome all of the rejections, stating only that the reports cited by appellants were accompanied by a publication by Anderson in 1998, that that "only anecdotal reports exist of patients being helped by gene therapy, and that there is no conclusive evidence of successful treatment of human disease." This is not the legal standard, however. All appellants must establish is that one can practice the claimed method. This they have done.

(c) Rejections Under 35 U.S.C. § 102

Claims 13, 15, 19, and 20 were rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,254,532 to Schwarz ("Schwarz").

i. The Legal Standard.

For a rejection of claims to be properly founded under 35 USC §102, it must be established that a prior art reference discloses each and every element of the claims. *Hybritech Inc v Monoclonal Antibodies Inc*, 231 USPQ 81 (Fed. Cir. 1986), *cert. denied*, 480 US 947 (1987); *Scripps Clinic & Research Found v Genentech Inc*, 18 USPQ2d 1001 (Fed. Cir. 1991). The Federal Circuit held in *Scripps*, 18 USPQ2d at 1010:

> Invalidity for anticipation requires that all of the elements and limitations of the claim are found within a single prior art reference. . . *There must be no difference* between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. (Emphasis added)

A reference that fails to disclose even one limitation will not be found to anticipate, even if the missing limitation could be discoverable through further experimentation. As the Federal Circuit held in *Scripps, Id.*:

> [A] finding of anticipation requires that all aspects of the claimed invention were already described in a single reference: a finding that is not supportable if it is necessary to prove facts beyond those disclosed in the reference in order to meet the claim limitations. The role of extrinsic evidence is to educate the decision-maker to what the reference meant to persons of ordinary skill in the field of the invention, not to fill in the gaps in the reference.

For a prior art reference to anticipate a claim, it must enable a person skilled in the art to practice the invention. The Federal Circuit held that "a §102(b) reference must sufficiently describe the claimed invention to have placed the public in possession of it. . . [E]ven if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling." *Paperless Accounting Inc v Bay Area Rapid Transit Sys.*, 231 USPQ 649, 653 (Fed. Cir. 1986) (citations omitted).

ii. The Prior Art.

Schwarz

Claim 13 defines a conjugate of an agent and a molecule to be delivered to a large vessel endothelial cell. Appellants respectfully submit that "binding selectively to endothelial protein C receptor...", simply characterizes the binding portion of the conjugate. One of ordinary skill in the art would have no problem concluding that "a molecule to be *delivered*..." (claim 13,

emphasis added), refers to a molecule that will be effectively *delivered* because it is *already conjugated* to the agent that selectively binds to the endothelial protein C receptor. The term "delivered" is typically interpreted to mean that something is "guided" to a particular location. The agent, in the present case, guides the molecule to the endothelial cell because the agent, conjugated to the molecule, binds selectively to the endothelial protein C receptor. The reason for "conjugating" a molecule to an agent, that has specificity to a particular receptor, is to ensure effective delivery. One would readily realize that administration of a random molecule to a cell, would not result in effective binding to the cell, internalization, translocation to the nucleus, and expression (if needed).

Schwarz teaches at column 2, lines 21-23, purified protein S in combination with activated protein C is immobilized at the surfaces of artificial vessels to prevent thrombosis. The Examiner has asserted that according to U.S. Patent No. 5,852,171 (to Fukudome *et al.*), protein S and activated protein C are inherently conjugated when bound to EPCR on large vessel endothelial cells. Fukudome teaches, at lines 45-47 of column 2, the binding of protein S to the APC/protein C on negatively charged surfaces, suggesting that APC/protein C must be on the negatively charged surface as a prerequisite for protein S binding. Fukudome does not teach the conjugation of protein S and protein C. Additionally, Schwarz teaches in Example 10 that protein S and protein C are added in a *combined application*, but do not teach a conjugation of protein S and protein C. The claims, as pending, are drawn to *a conjugate* that is selectively delivered to a large vessel endothelial cell. Neither Schwarz nor Fukudome teach a *conjugate of an agent and a molecule* to be *delivered* to a large vessel endothelial cell.

iii. The Examiner has completely failed to individually examine the dependent claims.

It is well established that each claim must be separately examined for patentability. It is not enough, as here, to look at a single independent claim and reject all claims. No rationale has been presented as to why a conjugate of an agent binding selectively to a endothelial protein C receptor and a protein (claim 20) would not be allowed in view of the specification or the prior art.

(9) SUMMARY AND CONCLUSION

In view of the foregoing discussions, the prior art fails to teach a method for the selective delivery of molecules to the nucleus of endothelial cells. The prior art is directed to the delivery of purified protein S *in combination with* activated protein C being immobilized at the surfaces of artificial vessels. There is no teaching of a *conjugate* of an agent binding selectively to endothelial protein C receptor (EPCR) and the molecule to be delivered.

The claims as pending are clearly enabled in view of the specification and state of the art at the time of filing. The specification and references that have been previously submitted (in particular, the Baumgartner *et. al.* reference) reveal (1) the state of the art at the time of filing and (2) that one of ordinary skill in the art would have reasonably expected that the claimed method, using molecules conjugated to agents that selectively bind to EPCR, could be effectively used *for the delivery* of nucleic acid to endothelial cells in need of treatment.

For the foregoing reasons, Appellant submits that all claims 1-25 are patentable.

Respectfully submitted,

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Date: May 28, 2002

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I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Patrea Pabst

Date: May 28, 2002

Appendix: Claims On Appeal

1. A method for selectively delivering molecules to the nucleus of endothelial cells of the large vessels, comprising

administering a conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) and the molecule to be delivered to large vessel endothelial cells, wherein the molecules are delivered to the nucleus of the large vessel endothelial cells.

2. The method of claim 1 wherein the conjugate is formed between the molecule to be delivered and an antibody to EPCR.

3. The method of claim 1 wherein the conjugate is formed between the molecule to be delivered and activated protein C.

4. The method of claim 1 wherein the conjugate comprises a chimeric antibody binding to the molecule to be delivered and to EPCR.

5. The method of claim 1 wherein the molecule to be delivered is a nucleic acid molecule and the nucleic acid molecule is a gene or cDNA under the control of a promoter expressed in the nucleus of an endothelial cell and the nucleic acid molecule is delivered by directly contacting the endothelial cells of large vessels with the nucleic acid molecule conjugate or by catherization to the endothelial cells.

6. The method of claim 5 wherein the nucleic acid molecule is selected from the group consisting of triplex forming oligonucleotides, ribozymes, guide sequences for ribozymes, and antisense.

7. The method of claim 1 wherein the molecule to be delivered is selected from the group consisting of drugs other than nucleic acids and proteins and diagnostic agents.

8. The method of claim 1 wherein the molecule to be delivered is a protein.

9. The method of claim 8 wherein the protein is a transcription factor.

10. The method of claim 1 wherein the molecule to be delivered is coupled to the agent which binds to EPCR by molecules selected from the group consisting of steptavidin and biotin, and molecules having multiple positive charges.

11. The method of claim 1 wherein the conjugate is administered to large vessel endothelial cells in culture or isolated from an individual.

12. The method of claim 1 wherein the conjugate is administered directly to the cells of to an individual in need of treatment or diagnosis.

13. A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

14. The conjugate of claim 13 wherein the conjugate is formed with an antibody to EPCR, or a fragment or recombinant molecule based thereon, binding to EPCR.

15. The conjugate of claim 13 wherein the conjugate is formed between the agent to be delivered and activated protein C.

16. The conjugate of claim 13 wherein the molecule to be delivered is a nucleic acid molecule in combination with means for directly contacting the nucleic acid molecule conjugate directly with the endothelial cells of large vessels, wherein the means are for in vitro treatment of the cells or by catherization to the endothelial cells.

17. The conjugate of claim 16 wherein the nucleic acid molecule is a gene or cDNA under the control of a promoter expressed in the nucleus of an endothelial cell.

18. The conjugate of claim 16 wherein the nucleic acid molecule is selected from the group consisting of triplex forming oligonucleotides, ribozymes, guide sequences for ribozymes, and antisense.

19. The conjugate of claim 13 wherein the molecule to be delivered is a drug other than nucleic acids and proteins.

20. The conjugate of claim 13 wherein the molecule to be delivered is a protein.

21. The conjugate of claim 20 wherein the protein is a transcription factor.

22. The conjugate of claim 20 comprising a coupling means which binds the molecule to be delivered to the agent which binds EPCR.

23. The conjugate of claim 22 wherein the coupling means is a positively charged polymer or molecule.

24. The conjugate of claim 22 wherein the coupling means is streptavidin-biotin.

25. The conjugate of claim 13 comprising a chimeric antibody which binds to EPCR and to the molecule to be delivered.

APPENDIX: Claims as Proposed to be Amended on Appeal

1. A method for selectively delivering molecules to the nucleus of endothelial cells of the large vessels, comprising

administering a conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) and the molecule to be delivered to large vessel endothelial cells, wherein the molecules are delivered to the nucleus of the large vessel endothelial cells.

2. The method of claim 1 wherein the conjugate is formed between the molecule to be delivered and an antibody to EPCR.

3. The method of claim 1 wherein the conjugate is formed between the molecule to be delivered and activated protein C.

4. The method of claim 1 wherein the conjugate comprises a chimeric antibody binding to the molecule to be delivered and to EPCR.

5. The method of claim 1 wherein the molecule to be delivered is a nucleic acid molecule and the nucleic acid molecule is a gene or cDNA under the control of a promoter expressed in the nucleus of an endothelial cell and the nucleic acid molecule is delivered by directly contacting the endothelial cells of large vessels with the nucleic acid molecule conjugate or by catherization to the endothelial cells.

6. The method of claim 5 wherein the nucleic acid molecule is selected from the group consisting of triplex forming oligonucleotides, ribozymes, guide sequences for ribozymes, and antisense.

7. The method of claim 1 wherein the molecule to be delivered is selected from the group consisting of drugs other than nucleic acids and proteins and diagnostic agents.

8. The method of claim 1 wherein the molecule to be delivered is a protein.

9. The method of claim 8 wherein the protein is a transcription factor.

10. The method of claim 1 wherein the molecule to be delivered is coupled to the agent which binds to EPCR by molecules selected from the group consisting of steptavidin and biotin, and molecules having multiple positive charges.

11. The method of claim 1 wherein the conjugate is administered to large vessel endothelial cells in culture or isolated from an individual.

12. The method of claim 1 wherein the conjugate is administered directly to the cells of to an individual in need of treatment or diagnosis.

13. A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

14. A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of an antibody to EPCR, or a fragment or recombinant molecule based thereon, binding to EPCR, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a

chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

15. A conjugate of activated protein C, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin between the molecule to be delivered and the activated protein C.

16. The conjugate of claim 13 wherein the molecule to be delivered is a nucleic acid molecule in combination with means for directly contacting the nucleic acid molecule conjugate directly with the endothelial cells of large vessels, wherein the means are for in vitro treatment of the cells or by catherization to the endothelial cells.

17. The conjugate of claim 16 wherein the nucleic acid molecule is a gene or cDNA under the control of a promoter expressed in the nucleus of an endothelial cell.

18. The conjugate of claim 16 wherein the nucleic acid molecule is selected from the group consisting of triplex forming oligonucleotides, ribozymes, guide sequences for ribozymes, and antisense.

19. The conjugate of claim 13 wherein the molecule to be delivered is a drug other than nucleic acids and proteins.

20. The conjugate of claim 13 wherein the molecule to be delivered is a protein.

21. A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with

EPCR and fragments thereof binding to EPCR, and a a transcription factor, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

22. A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a protein, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin and comprising a coupling means which binds the protein to be delivered to the agent which binds EPCR.

23. The conjugate of claim 22 wherein the coupling means is a positively charged polymer or molecule.

24. The conjugate of claim 22 wherein the coupling means is streptavidin-biotin.

25. A conjugate of a chimeric antibody which binds to endothelial protein C receptor (EPCR) and a molecule to be delivered, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

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ATL1 #524605 v1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Charles T. Esmon and Jun Xu

Serial No.: 09/139,425 Art Unit: 1636

Filed: August 25, 1998 Examiner: W. Sandals

For: *"TARGETING OF MOLECULES TO LARGE VESSEL ENDOTHELIUM USING PCR"*

Assistant Commissioner for Patents
Washington, D.C. 20231

AMENDMENT

Sir:

Please amend the claims as follows. The claims that were objected to as being dependent on independent claims have been re-written in independent form.

In the Claim

14. (amended) [The conjugate of claim 13 wherein the conjugate is formed with] A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of an antibody to EPCR, or a fragment or recombinant molecule based thereon, binding to EPCR, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

15. (amended) [The conjugate of claim 13] A conjugate of activated protein C, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin [wherein the conjugate is formed] between the [agent] molecule to be delivered and the activated protein C.

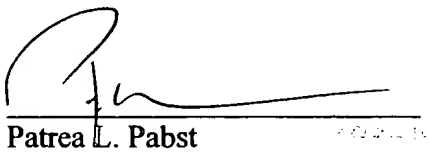
21. (amended) [The conjugate of claim 20] A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a a transcription factor, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin [wherein the protein is a transcription factor].

22. (amended) [The conjugate of claim 20] A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a protein, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin and comprising a coupling means which binds the [molecule] protein to be delivered to the agent which binds EPCR.

25. (amended) [The conjugate of claim 13] A conjugate of a chimeric antibody which binds to endothelial protein C receptor (EPCR) and a molecule to be delivered, wherein the conjugate

is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

Respectfully submitted,



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Appendix: Marked Up Copy of Claims On Appeal

1. A method for selectively delivering molecules to the nucleus of endothelial cells of the large vessels, comprising

administering a conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) and the molecule to be delivered to large vessel endothelial cells, wherein the molecules are delivered to the nucleus of the large vessel endothelial cells.

2. The method of claim 1 wherein the conjugate is formed between the molecule to be delivered and an antibody to EPCR.

3. The method of claim 1 wherein the conjugate is formed between the molecule to be delivered and activated protein C.

4. The method of claim 1 wherein the conjugate comprises a chimeric antibody binding to the molecule to be delivered and to EPCR.

5. The method of claim 1 wherein the molecule to be delivered is a nucleic acid molecule and the nucleic acid molecule is a gene or cDNA under the control of a promoter expressed in the nucleus of an endothelial cell and the nucleic acid molecule is delivered by directly contacting the endothelial cells of large vessels with the nucleic acid molecule conjugate or by catherization to the endothelial cells.

6. The method of claim 5 wherein the nucleic acid molecule is selected from the group consisting of triplex forming oligonucleotides, ribozymes, guide sequences for ribozymes, and antisense.

7. The method of claim 1 wherein the molecule to be delivered is selected from the group consisting of drugs other than nucleic acids and proteins and diagnostic agents.

8. The method of claim 1 wherein the molecule to be delivered is a protein.

9. The method of claim 8 wherein the protein is a transcription factor.

10. The method of claim 1 wherein the molecule to be delivered is coupled to the agent which binds to EPCR by molecules selected from the group consisting of steptavidin and biotin, and molecules having multiple positive charges.

11. The method of claim 1 wherein the conjugate is administered to large vessel endothelial cells in culture or isolated from an individual.

12. The method of claim 1 wherein the conjugate is administered directly to the cells of to an individual in need of treatment or diagnosis.

13. A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

14. (amended) [The conjugate of claim 13 wherein the conjugate is formed with] A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of an antibody to EPCR, or a fragment or recombinant molecule based thereon, binding to EPCR, and a molecule to be delivered to a large vessel endothelial cell,

wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

15. (amended) [The conjugate of claim 13] A conjugate of activated protein C, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin [wherein the conjugate is formed] between the [agent] molecule to be delivered and the activated protein C.

16. The conjugate of claim 13 wherein the molecule to be delivered is a nucleic acid molecule in combination with means for directly contacting the nucleic acid molecule conjugate directly with the endothelial cells of large vessels, wherein the means are for in vitro treatment of the cells or by catherization to the endothelial cells.

17. The conjugate of claim 16 wherein the nucleic acid molecule is a gene or cDNA under the control of a promoter expressed in the nucleus of an endothelial cell.

18. The conjugate of claim 16 wherein the nucleic acid molecule is selected from the group consisting of triplex forming oligonucleotides, ribozymes, guide sequences for ribozymes, and antisense.

19. The conjugate of claim 13 wherein the molecule to be delivered is a drug other than nucleic acids and proteins.

20. The conjugate of claim 13 wherein the molecule to be delivered is a protein.

21. (amended) [The conjugate of claim 20] A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a a transcription factor, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin [wherein the protein is a transcription factor].

22. (amended) [The conjugate of claim 20] A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a protein, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin and comprising a coupling means which binds the [molecule] protein to be delivered to the agent which binds EPCR.

23. The conjugate of claim 22 wherein the coupling means is a positively charged polymer or molecule.

24. The conjugate of claim 22 wherein the coupling means is streptavidin-biotin.

25. (amended) [The conjugate of claim 13] A conjugate of a chimeric antibody which binds to endothelial protein C receptor (EPCR) and a molecule to be delivered, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

APPENDIX: Clean Copy of Claims on Appeal

1. A method for selectively delivering molecules to the nucleus of endothelial cells of the large vessels, comprising

administering a conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) and the molecule to be delivered to large vessel endothelial cells, wherein the molecules are delivered to the nucleus of the large vessel endothelial cells.

2. The method of claim 1 wherein the conjugate is formed between the molecule to be delivered and an antibody to EPCR.

3. The method of claim 1 wherein the conjugate is formed between the molecule to be delivered and activated protein C.

4. The method of claim 1 wherein the conjugate comprises a chimeric antibody binding to the molecule to be delivered and to EPCR.

5. The method of claim 1 wherein the molecule to be delivered is a nucleic acid molecule and the nucleic acid molecule is a gene or cDNA under the control of a promoter expressed in the nucleus of an endothelial cell and the nucleic acid molecule is delivered by directly contacting the endothelial cells of large vessels with the nucleic acid molecule conjugate or by catherization to the endothelial cells.

6. The method of claim 5 wherein the nucleic acid molecule is selected from the group consisting of triplex forming oligonucleotides, ribozymes, guide sequences for ribozymes, and antisense.

7. The method of claim 1 wherein the molecule to be delivered is selected from the group consisting of drugs other than nucleic acids and proteins and diagnostic agents.

8. The method of claim 1 wherein the molecule to be delivered is a protein.

9. The method of claim 8 wherein the protein is a transcription factor.

10. The method of claim 1 wherein the molecule to be delivered is coupled to the agent which binds to EPCR by molecules selected from the group consisting of steptavidin and biotin, and molecules having multiple positive charges.

11. The method of claim 1 wherein the conjugate is administered to large vessel endothelial cells in culture or isolated from an individual.

12. The method of claim 1 wherein the conjugate is administered directly to the cells of to an individual in need of treatment or diagnosis.

13. A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

14. A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of an antibody to EPCR, or a fragment or recombinant molecule based thereon, binding to EPCR, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a

chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

15. A conjugate of activated protein C, and a molecule to be delivered to a large vessel endothelial cell, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin between the molecule to be delivered and the activated protein C.

16. The conjugate of claim 13 wherein the molecule to be delivered is a nucleic acid molecule in combination with means for directly contacting the nucleic acid molecule conjugate directly with the endothelial cells of large vessels, wherein the means are for in vitro treatment of the cells or by catherization to the endothelial cells.

17. The conjugate of claim 16 wherein the nucleic acid molecule is a gene or cDNA under the control of a promoter expressed in the nucleus of an endothelial cell.

18. The conjugate of claim 16 wherein the nucleic acid molecule is selected from the group consisting of triplex forming oligonucleotides, ribozymes, guide sequences for ribozymes, and antisense.

19. The conjugate of claim 13 wherein the molecule to be delivered is a drug other than nucleic acids and proteins.

20. The conjugate of claim 13 wherein the molecule to be delivered is a protein.

21. A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with

EPCR and fragments thereof binding to EPCR, and a a transcription factor, wherein the molecule is not a diagnostic label, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.

22. A conjugate of an agent binding selectively to endothelial protein C receptor (EPCR) selected from the group consisting of protein C, activated protein C, antibodies reactive with EPCR and fragments thereof binding to EPCR, and a protein, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin and comprising a coupling means which binds the protein to be delivered to the agent which binds EPCR.

23. The conjugate of claim 22 wherein the coupling means is a positively charged polymer or molecule.

24. The conjugate of claim 22 wherein the coupling means is streptavidin-biotin.

25. A conjugate of a chimeric antibody which binds to endothelial protein C receptor (EPCR) and a molecule to be delivered, wherein the conjugate is a chemical conjugate, fusion protein or conjugate formed by indirect binding by a positively charged polymer, chimeric antibody or streptavidin.